PROTEIN DIFFERENCES ASSOCIATED WITH THE LOSS OF MYELINATED AXONS AND FIBRILLARY GLIOSIS IN RAT OPTIC NERVES FOLLOWING WALLERIAN DEGENERATION

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Received 19 December 1974

1. Introduction

The overall polypeptide composition of the optic nerves of the rat was studied by SDS-gel electrophoresis in normal rats and in blind rats 5 months after removal of the eyes. Apart from the disappearance of myelin proteins after long-standing Wallerian degeneration, the disc gel pattern of normal and degenerated optic nerves was remarkably similar, suggesting that proteins of nerve and glial fibers have similar mol. wts.

The histological complexity of the central nervous system is a major difficulty to interpret descriptive surveys of brain proteins and the comparison of normal and pathological tissues with selective loss of cell constituents has provided a useful approach to the problem [1,2]. In this report we have utilized Wallerian degeneration of the rat optic nerves as an experimental model to study the polypeptide composition of axons and glial fibers. Striking changes occur in myelinated tracts, including the optic nerves, in the course of the degeneration observed in the peripheral segment of the axon separated from the nutrient cell body. At the end stage of the process the tracts are remarkably shrunken and grayish in color. Microscopically, the myelinated nerve fibers have disappeared and are replaced by astroglial processes packed with filaments [3,4]. This increase in astroglial fibers is partly real and reactive in nature (fibrillary gliosis), partly secondary to atrophy of the tissue with condensation of the normal supportive astroglial framework.

2. Materials and methods

Wallerian degeneration of the rat optic nerves was produced in 3-month-old Sprague-Dawley rats by enucleation of the eyes. The rats were sacrificed 5 months after enucleation, at a time when axons have disappeared. In each experiment 4 optic nerves (normal or degenerated) were pooled. The nerves were homogenized immediately after removal in 400 μl of 1% SDS, 1.5% dithiothreitol and 8% sucrose in the running buffer used for electrophoresis. The homogenates were boiled for 3 min. Preliminary experiments using different heating times (30 sec up to 10 min) with and without dithiothreitol had shown these to be the optimal conditions for the solubilizations of proteins, as indicated by the staining of bands on SDS–acrylamide gels, and specifically of the glial fibrillary acidic (GFA) protein, as shown by the immunodiffusion titer. SDS-gel electrophoresis was performed according to Shapiro, Viñuela and Maizel [5] using 30 μl of homogenate per gel. The gels contained 7.5% acrylamide, 0.2% cross-linker, 0.025 M phosphate buffer pH 7.3 and 0.1% SDS. Electrophoresis was conducted at room temperature at 1.9 mA per tube for 30 min followed by 3 mA per tube till the tracking dye (Bromphenol Blue) reached the lower end of the gel. The gels were stained overnight with Fast Green (1% in 10% acetic acid). Ouchterlony double diffusion and disc immunodiffusion were performed as described before [6]. Antisera

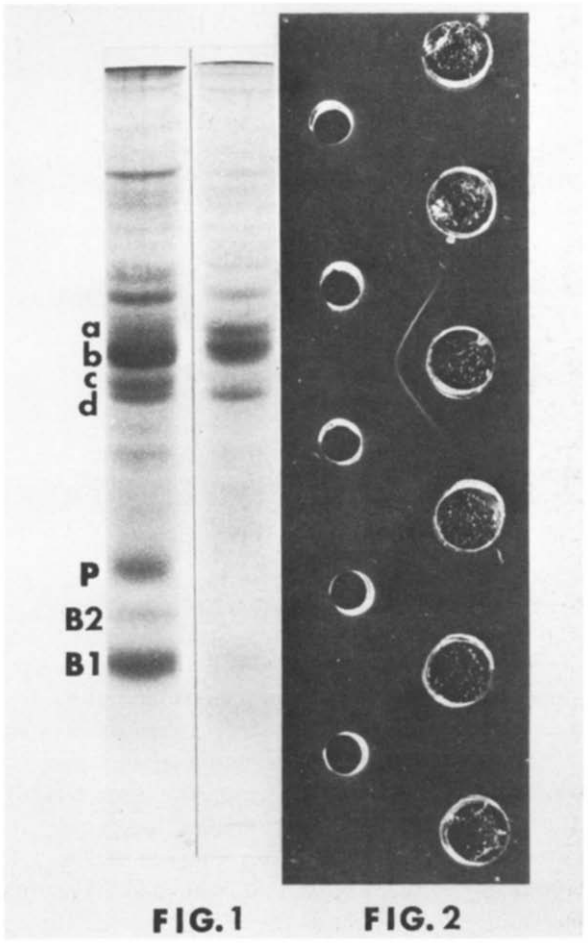

FIG. 1 FIG. 2

Fig.1. SDS-gel electrophoresis at 7.5% acrylamide concentration of normal (gel 1) and degenerated rat optic nerves 5 months after enucleation of the eyes (gel 2). Identified bands are: a, 57 000; b, 52 000; c, 47 000; d, 45 000; P (proteolipid protein of myelin), 22 5000; B_2 (large basic protein of rat myelin), 18 500; B_1 (small basic protein of rat myelin). Note the disappearance of bands c, P, B_2 and B_1 in the degenerated optic nerves.

Fig.2. Disc immunodiffusion of degenerated optic nerves 5 months after enucleation of the eyes. The wells on the left contain GFA protein antiserum. The wells on the right contain 2 mm slices of a 7.5% acrylamide gel frozen immediately following electrophoresis in SDS. Disc immunodiffusion is positive in slices 14, 15 and 16, with slice 15 giving the strongest precipitin line (only this line is visible in the photograph). The position of the slices positive by disc immunodiffusion is indicated on the densitometric tracing of a stained SDS–acrylamide gel from the same electrophoretic preparation (see fig.3).

against 2 different preparations of GFA protein previously obtained in New Zealand albino rabbits were used [7]. The following proteins were used as references for mol. wt determination: conalbumin (76 600), serum albumin (68 000), ovalbumin (43 000), chymotrypsinogen A (25 700).

3. Results

The protein patterns of normal and degenerated optic nerves were extremely reproducible and are illustrated in figs. 1 and 3. Bands migrating within the range of proteolipid protein (band P, 22 500) and of the larger basic protein of rat myelin (band B_2, 18 500) were not recognizable in degenerated nerves. The mol. wt of a faster migrating band which had disappeared in blinded rat, probably corresponding to the smaller basic protein of rat myelin (band B_1), could not be determined. Proteins migrating in this range were not on a straight line when log mol. wt was plotted versus mobility. A thin band at 47 000 (band d) was also missing in blinded rats. Band a (57 000) and band b (52 000) were more distinctly separated in degenerated optic nerves than in normal ones. By disc immunodiffusion with GFA protein antiserum (fig.2), the precipitin band extended from band b to band c in normal optic nerves; from band b to band d in degenerated nerves. In both cases the precipitin line was stronger in the gel slice containing band b (fig.3).

4. Discussion

The absence of bands migrating within the range of myelin proteins in optic nerves 5 months after enucleation of the eyes is well in keeping with electron microscopic data showing almost complete disappearance of myelin in long-standing Wallerian degeneration [3,4]. It is possible that the band at 47 000 which was missing in blinded rats also represents a myelin component. The absence of a band at about 45 000 has been observed in quaking mice [2], a mutation characterized by myelin deficiency in the central nervous system with preservation of axons [8].

Apart from the disappearance of myelin proteins after long-standing degeneration, the electrophoretograms of whole optic nerve extracts from normal and

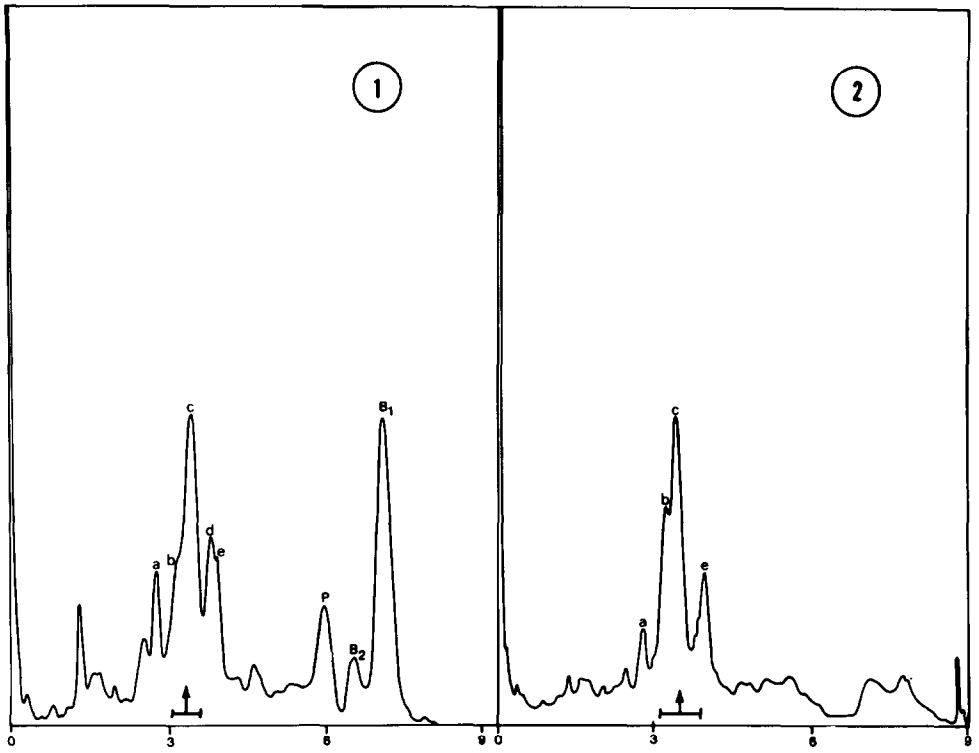

Fig.3. Densitometric tracings of normal (1) and degenerated optic nerves (2) on SDS–acrylamide gels (gels 1 and 2 in fig.1). The gels were scanned at 620 nm with a Gilford Model 2400S spectrophotometer. Baseline of arrow indicates area of positive disc immunodiffusion; point of arrow indicates strongest precipitin line.

blinded rats were similar, and no other correlation could be made between changes in tissue constituents and variations in the electrophoretic pattern. This could be interpreted as indicating that nerve and glial fibers are similar in polypeptide composition. According to Johnson and Sinex [9] neurotubules and astroglial filaments are composed of the same or very similar protein subunits, that is tubulin. A more likely explanation may reside in the fact that the protein subunits of two major fibrillary structures of the brain, that is neurofilaments and glial filaments, although chemically different have similar molecular weight and thus may be difficult to separate on SDS-gel electrophoresis. A mol. wt of 51 000 has been estimated by SDS-gel electrophoresis for the protein of neurofilaments [10]. GFA protein, a major constituent of glial fibers, has been separated into closely related polypeptides ranging in mol. wt from 54 000 to 40 500 [11], the higher mol. wt species being the major component in some preparations from normal human brain and in bovine brain (in preparation). The higher molecular weight polypeptides are degraded in the tissue, but not in purified preparations, into the lower molecular weight species in the 45 000–40 500 range [11]. The relative stability to degradation of the lower mol. wt species may account for the fact that they were the major constituents of GFA protein previously isolated from human brains with multiple sclerosis plaques [6,12]. It may be noted that the optic nerves were immediately dissolved in dithiothreitol–SDS after removal and that breakdown was not likely to occur in these conditions.

Acknowledgements

Supported by Research Grants of the United States Public Health Service (NS-11073) and of the Veterans Administration.

References

[1] Mallet, J., Huchet, M., Shelanski, M. and Changeux, J. P. (1974) FEBS Lett. 46, 243–246.
[2] Vaughan, W. J. and Fliesler, S. J. (1974) J. Neurochem. 23, 279–281.
[3] Bignami, A. and Ralston, H. J. (1969) Brain Res. 13, 444–461.
[4] Vaughn, J. E. and Pease, D. C. (1970) J. Comp. Neur. 140, 207–226.
[5] Shapiro, A. L., Viñuela, E. and Maizel J. B. (1967) Biochem. Biophys. Res. Commun. 28, 815–820.
[6] Dahl, D. and Bignami, A. (1973) Brain Res. 57, 343–360.
[7] Bignami, A. and Dahl, D. (1974) J. Comp. Neur. 153, 27–38.
[8] Sidman, R. L., Dickie, M. M. and Appel, S. H. (1964) Science 144, 309–311.
[9] Johnson, L. and Sinex, F. M. (1974) J. Neurochem. 22, 321–326.
[10] Iqbal, K., Wisniewski, H. M., Shelanski, M. L., Brostoff, S. Liwnicz, B. H. and Terry, R. D. (1974) Brain Res. 77, 337–343.
[11] Dahl, D. and Bignami, A. (1975) Biochim Biophys. Acta, in press.
[12] Uyeda, C. T., Eng, L. F. and Bignami, A. (1972) Brain Res. 37, 81–89.